POTENTIAL USE OF SYSTEMIC ACARICIDES IN THE CONTROL OF CHIGGER (ACARINA: TROMBICULIDAE) POPULATIONS ON RODENTS

Ву

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DEDICATION

I dedicate this dissertation to my wife, $\label{eq:JEAN} {\sf JEAN}$

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Abstract of Dissertation Presented to the Graduate Council of the University of Florida in Partial Fulfillment of the Requirements for the Degree of Doctor of Philosophy

POTENTIAL USE OF SYSTEMIC ACARICIDES IN THE CONTROL OF CHIGGER (ACARINA: TROMBICULIDAE) POPULATIONS ON RODENTS

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In a preliminary study of the chigger species within the study area, six species were reported new to the State of Florida. Of 19 insecticides tested, only dimethoate was found to cause death to chiggers by systemic activity at the levels tested. Virtually 100 per cent mortality was found to occur in chiggers (Eutrombicula alfredducesi [Oudemans]) fed on guinea pigs treated at a level of 75 mg/kg or more, while chiggers fed on guinea pigs feeding on baits treated with 0.1 per cent or higher level of dimethoate produced 100 per cent kill. Dimethoate treated bait, fed to cotton rats (Sigmodon hispidus) at doses of 0.1 per cent or higher, produced virtually 100 per cent kill of the chiggers applied. There was no apparent change in chigger activity, as indicated by carbon dioxide production, when chiggers were exposed to varying wavelengths of the electromagnetic spectrum.

INTRODUCTION

Chiggers or trombiculid larvae have long been known to be a problem to both man and animals, either as vectors of disease or as causes of extreme irritation due to their mode of feeding. A few species of chiggers in Asia transmit the rickettsial disease, scrub typhus (Rickettsia tsutsugamushi), while quite a number of species throughout the world are known to attack man and cause an itching sensation and swelling at the site of their feeding. Four species of the genus Eutrombicula splendens (Ewing), alfreddugesi (Oudemans), batatas (Linnaeus) and belkeni (Gould) readily feed on man in North America. In addition the larvae of Neoschongastia americana (Hirst) cause considerable damage to domestic turkeys in several regions of the United States (Everett et al., 1973).

Major interest in the study of trombiculid mites was initiated with the occupation of the Far East by United States and Allied forces during the World War II. Scrub typhus was severe among a large number of troops, and in certain areas was even more important than malaria. Following wartime research in Burma, studies began to spread throughout the world, particularly in Malaysia (Audy, 1956; Traub and Frick, 1950; Traub $et\ aZ.$, 1954), the United States (Wharton and Fuller, 1952; Jenkins, 1948a, 1948b, 1949a, and 1949b), and England (Radford, 1954; Richards, 1950a and 1950b; Jones, 1950a, 1950b, and 1950c).

The chigger is the only pathopherous agent of scrub typhus in the Far East. As only the larvae feed on animals, the disease must be

transmitted both transovarially and transtadially in order for it to be maintained (Rapmund et al., 1969; Rapmund et al., 1972). The primary hosts of this disease in the Far East are rodents. Normal removal of the host, either by trapping, shooting or poisoning, does not eliminate the disease, but actually increases the chance that man will come into contact with the chiggers that transmit this disease.

The ideal solution for the control of scrub typhus within a given area would be to eliminate both the chiggers and their hosts.

As removal of the host would increase the probability of man contracting the disease, the same recommendation that is used for the control of plague can be made for the control of scrub typhus; that is, the vector should be controlled before the hosts are eliminated.

Previous chemical control of chiggers has relied on the spraying of acaricides in suspected habitats. Such operations are feasible in areas such as the grassland habitat of Leptotrombidium (Leptotrombidium) akamushi (Brumpt), but not in the densely forested areas where L. (L.) deliense (Walch) is often found.

The primary objective of this research is to determine the feasibility of utilizing an acaricide as a systemic for controlling chiggers. The acaricide must effectively eliminate the vector chigger and cause little or no effect to the host, since, as previously indicated, this could cause increased problems. The ideal method of administering such a compound would be by placing it in the hosts' food by means of bait stations within their harborage or nesting areas.

LITERATURE REVIEW

Life History

Classically, the term chigger has been used in reference to the six-legged larvae of the family Trombiculidae. Currently, the term may be used for all stages of this family (Audy, 1968). The following is the phylogenic breakdown to family as determined by Krantz (1970):

Class	Arachnida
Subclass	Acari
0rder	Acariformes
Suborder	Prostigmata
Supercohort	Parasitengona
Superfamily	Trombidioidea
Family	Trombiculidae

Wharton and Fuller (1952) present the following diagnosis for the family Trombiculidae:

Trombidiform mites whose larvae have chelicera with two segments, the basal segment stout and muscular, the distal segment a sclerotized, curved blade with or without tooth-like projections. Each palp has five segments, the basal one of which, on the two palpi, fuse along the midline and form a median, anterior, laminar projection that extends beyond the basal segment of the chelicerae; this projection bears a pair of lateral wings, or galeae, that curl dorsal about the chelicerae, and each galea bears a sublateral seta near the apex; each basal segment also bears a strong seta just posterior to the junction with the palpal femur; the second palpal segment, or femur, bears a single seta; the fourth, or

tibia, has three setae (one dorsal, one lateral, and one ventral) and a terminal, palpal claw; the fifth, or tarsus, articulates ventrally with the tibia and opposes the palpal claw in thumb-like fashion; it bears several setae (usually eight), the basal one of which is a striated, sensory seta. The body is usually red in color but may be almost colorless; it bears a dorsal plate or scutum at the level of the anterior two pairs of legs, usually two pairs of eyes that flank the scutum, several rows of dorsal setae, several rows of ventral setae, occasionally a posterior plate or a posterior group of specialized setae, a ventral anus, three pairs of legs, an urstigma or sclerotized pit associated with the posterior distal angle of coxa I, and at times a pair of tracheal trunks that open through stigmata in the region of the gnathosoma. The scutum bears from three to six marginal, scutal setae (or infrequently more) and a pair of pseudostigmata from which the sensillae or pseudostigmatic organs arise. The legs are composed of six segments if the femur is undivided and of seven if the femur consists of a basifemur and telofemur. (p. 41)

Life history studies have been generated primarily from three different geographical areas, with studies of the specific chiggers in relation to their importance in these areas. These include 1) the scrub typhus vectors of the Far East, 2) the pest chiggers of man of North and Central America, and 3) the pestiferous "harvest" mite of England. Obviously, the life histories of these are similar, differing primarily in length of time within each stage.

Seven definite stages are known to occur within the life cycle of trombiculid mites. These include: egg, deutovum, larva, nymphochrysalis, nymph, imagochrysalis, and adult. The nymphochrysalis, an inactive stage between the larva and nymph, has also been referred to by some authors as either the prenymph or nymphophane, while the imagochrysalis, a similar stage between the nymph and adult, has been called a preadult or telieophane. Table 1 lists the nomenclature used by various authors in the literature on chiqgers.

Nomenclature of the developmental stages of Trombiculid mites as used by various authors. Table 1.

Wharton and Fuller (1952)	Johnston and Wacker (1967)	Cocklings (1948)	Jones (1954) Neal and Barnett (1961)	Michener (1946a)
Egg	Egg	Egg	Egg	Egg
Deutovum	Prelarva	Deutovum	Deutovum	Deutovum
Larva	Larva	Larva	Larva	Larva
Nymphochrysalis	Protonymph	Nymphophane	Prenymph	Protonymph .
Nymph	Deutonymph	Nymph	Nymph	Nymph
Imagochrysalis	Tritonymph	Telieophane	Preadult	Preadult
Adult	Adult	Adult	Adult	Adult
		The second secon		

Sasa (1961) reviews much of the earlier work on the biology and life history of chiggers. Many of these early studies were conducted on the vectors of scrub typhus in Japan. Ewing (1944), Michener (1946a), Melvin (1946), Jenkins (1948a), and Wharton and Fuller (1952) presented early findings of the life history of chiggers attacking man in America. The life cycle of the scrub typhus chigger, Leptotrombidium (Leptotrombidium) akammushi (Brumpt) was studied by Neal and Barnett (1961). Post developmental studies of Eutrombicula splendens (Ewing) were conducted by Johnston and Wacker (1967).

The life cycle of the chigger is fairly complicated as compared with most other mites. Descriptions of the stages of the life cycle are made in following paragraphs. The egg is deposited by the adult female in the soil or litter. After development the egg splits, leaving an inactive deutovum. The larva emerges from the deutovum, moving onto leaves or grasses, and awaits for a vertebrate host. The larva is the only stage that actively parasitizes a vertebrate animal. Upon becoming fully engorged the larva drops to the ground and develops into an inactive nymphochrysalis, afterward emerging into an active nymph. After feeding sufficiently on arthropods or their eggs, the nymph then develops into another inactive stage, the imagochrysalis. Following this developmental stage, the adult emerges. Table 2 lists the developmental times for six species of trombiculid mites.

Eggs

The amber yellow to pale yellow spherical eggs are laid singly.

These become ovoid with development of the embryo. With a laboratory

Table 2. Summary of developmental times of six species of Trombiculid mites, expressed in days.

	Eutrombicula splendens*	Eutrombicula alfreddugesi*	Eutrombicula batatas	Euschoengastia indica#	Leptotrombidium akamusht	Neoschongastia americana (at 32°C)
Egg	6	6	5*	6	7-11	7.6
Deutovum	7	7	7*	6	7-13	7.1
Larva		(Time de	pends on	availabil	ity of hos	ts)
Nymphochrysalis	6	6	5-7°	4-6	4-12	7.5
Nymph**	6	6	6*	4	6-28	15.6
Imagochrysalis	6	6	5-7°	6	4-11	7.5

^{*}Jenkins (1947) #Wharton (1946)

[°]Michener (1946a) +Neal and Barnett (1961)

^{&#}x27;Everett, Price and Kunz (1973)

^{**}Whifed nymphs can live for long periods of time without suitable food [i.e. E. batatas 45 days; Michener (1946a)].

culture of L. (L.) akamushi the daily egg production per female varied from 2.4 to 21.7, with one female producing a maximum of 41 eggs on a single day (Neal and Barnett, 1961). Egg development took from 7 to 11 days, with a mean of 8 days.

Deutovum

Upon complete development of the egg, the egg ruptures exposing a quiescent instar, the deutovum. This stage is hexapodal with the appendages regressive and setae lacking on the idiosome and appendages (Johnston and Wacker, 1967).

Larva

The larvae are round to oval hexapods, characteristically having a dorsal scutum, with a pair of prominent sensillae. This is the only stage that is parasitic on vertebrates and thus has received the most attention taxonomically. Due to the small number of adults that have been correlated with the larvae, identification of species within the family Trombiculidae is based upon larval characteristics. An example of this is found in the monograph by Womersley (1952) where he lists 221 species of larvae and only correlates 46 with nymphs or adults.

The white to reddish larva, upon transformation from the deutoval stage, usually rests on leaves or litter awaiting the arrival of a host. On the host it finds a suitable site for attachment (i.e. ears, around the eyes, anus or base of the legs). It then penetrates the host with its chelicerae and forms a stylosome (feeding tube) within the host tissue by the release of enzymes from the chigger (Cross, 1962). Neal and Barnett (1961) found that unfed larvae of L. (L.) akamushi survived

for a long period of time, 26 to 102 days. The rate of engorgement varies between species of chiggers and depends to a large degree upon which species of host that the chiggers feed on. L. (L.) akaamushi feeding on various laboratory rodents and White Leghorn chicks took approximately 40 hours for engorgement. Engorgement of E. spendens and E. alfreddugesi (Oudemens) feeding on reptiles in Florida usually took 8 days during May, 4-5 days during July and August and 13 days during September, while the maximum time observed to engorge on an indigo snake was 48 days (Jenkins, 1948a).

Normal engorgement increases the size of most chiggers by approximately 20 times. However, in some chiggers, notably of the genera Vatacarus and Riedlinia, neosomy ccurs (Audy et al., 1972). Larvae of Vatacarus, a parasite of the lungs of sea snakes (Laticuada spp.) in the western Pacific, increase in size by as much as 1,500 times or more, while Riedlinia, a parasite of bat wings, may enlarge up to 750 times.

Nymphochrysalis

The nymphochrysalis is a quiescent stage, having eight legs with all appendages regressive and lacking setae on the idiosoma. Stasis takes place entirely within the cuticle of the preceding larval instar (Johnston and Wacker, 1967). Formation of the nymphal appendages takes place within the regressive appendages of the nymphochrysalis. Rupture

 n_{eosomy} —the formation of new external morphological structure or taxonomically significant enlargement, resulting at least in part from the secretion of new cuticle, during a single active stadium or an invertebrate in a group that normally changes in external form only through a molt (Audy et az., 1972).

of the nymph from the larval skin often leaves the skin so intact that it can be used for taxonomic purposes, thus facilitating correlation of larvae with nymphs.

Nymphs

The nymph is an active, highly pilose octopod. The idiosoma is usually constricted between the proterosoma and hysterosoma, giving it a distinct figure-eight appearance. The nymph is predaceous on small soft-bodied arthropods or their eggs. In laboratory cultures, collembola and the eggs of certain mosquitoes have proved satisfactory as a food for rearing.

Imagochrysalis

The imagochrysalis is a quiescent stage, having eight legs and regressive appendages, and is without setae on the idiosoma. Like the nymphochrysalis, the stasis of this stage takes place entirely within the cuticle of the previous instar (Johnston and Wacker, 1967).

Adult

The adult, like the nymph, is a predaceous octopod with its appendages normally segmented and movable. It is more highly setaceous than the nymph. The adult has three pairs of genital papillae as opposed to the two pairs of the nymph (Johnston and Wacker, 1967).

Male adults of L. (L.) akamushi in a laboratory colony lived for an average of 116 days (range 2 to 332), while females of the same species lived for an average of 185 days (range 19 to 443) (Neal and Barnett, 1961). The females within this colony laid eggs for an

average of 75 days (range 8 to 243). In a study of 16 females from the above colony, an average of 900 eggs (range 236 to 2,405) was produced per female, with an average daily egg production of 8.5 per female (range 2.4 to 21.7). Melvin (1946) reported that a Eutrombicula sp. produced a cluster of 56 eggs overnight.

Lipovsky et al. (1957) described the mode of insemination of adult trombiculid mites. Copulation does not occur, but rather a spermatophore (a flexible stalk with a sperm sac attached) is deposited on the substrate. This resembles the fruiting bodies of various molds. Insemination occurs when the female comes in contact with the spermatophore. The female, while walking over the spermatophore, closes her genital plates, pulling the sperm sac from the stalk. A male need not be present for insemination, and neither does a female need to be present for deposition of the spermatophore.

Kaufmann and Traub (1966) reported parthenogenesis occurring within a laboratory colony of L. (L.) arenicola Traub. The parthenogenetic offspring were indistinguishable from ordinary chiggers and developed into both male and female adults, a deuterotokous form of parthenogenesis. However, only one-third of the parthenogenetic offspring developed to the larval stage, while nearly all the eggs of the normal adults developed into chiggers.

Mitchell and Nadchatram (1969) described the interesting method of defecation that occurs within adult trombiculid mites. As a splitting of the idiosoma is involved, the term "schizeckenosy" was coined. The "anus" of the mite is actually a blind excretory tube and thus is nonfunctional. Indigestible residues of food become packed in gut cells.

These gut cells become storage cells of feces or fecal lobes. As these lobes become packed with feces, they put the postero-dorsal part of the body under high tension, causing the body to split open and extrude the fecal lobe either completely or partially. In a natural situation, with the mite in the soil, the fecal lobe would be brushed off by soil or litter particles. In clusters, the fecal lobe may stick to glass containers causing death to the mite.

Biology and Habitats

Numerous early studies on the biology and habitats of the chiggers of Asia were conducted by Japanese workers. Sasa (1961) in his "Biology of Chiggers" reviews this early work. More recent studies of vectors of scrub typhus in Malaysia have been conducted by Harrison and Audy (1951, 1956) Hubert and Baker (1963a, 1963b) and Dohany et al. (to be published). Traub and Wisseman (1968a, 1968b) discuss in detail the habits of the vector mites and the epidemiology of scrub typhus. Distribution and biological studies of the harvest mite, Neotrombicula autummalis (Shaw), in Great Britain were conducted by Richards (1950b), Jones (1950a, 1950b) and Cockings (1948).

Distribution and biology of the chiggers affecting man in the United States was studied by Jenkins (1948a, 1949a, 1949b). Three species were found to occur in the eastern United States. Eutrombicula alfreddugesi was reported throughout the eastern and central United States, south to Florida and west to California. This species has also been reported from Central America, and south to Argentina in South America. Eutrombicula spendens occurs along the coastal areas

from Texas to New England, as well as in isolated locations of Michigan and Minnesota. Loomis (1956) reported *E. splendens* also occurring in Kansas. *Eutrombicula batatus* (Linnaeus) is a tropical chigger occurring in the warmer parts of the Southeastern United States, California and south into Venezuela in South America.

Gould (1950, 1956) reports that *E. belkini* (Gould) attacks man in California. He also lists one specimen from a snake (*Pituophis caterifer deserticola*) from Utah.

Seasonality

Wharton and Fuller (1952) point out that, under favorable conditions of a laboratory and often in tropical climes, chiggers may reproduce continuously. However, this is not the more common situation in nature. In studies at Duke University, ten generations of *E. splendens* were obtained within a one-year period. Wharton and Fuller (1952) point out that probably temperature affects the number of generations within a temperate climate, and possibly humidity within a tropical climate.

Jenkins (1948a) conducted extensive studies on the seasonality of the chiggers affecting man in the United States. He produced a map of the United States showing that chiggers within the southern tip of Florida were active throughout the entire year, while the chiggers within the northern-most part of their range within the United States were active only from 4 July to 10 September. Farrell (1956) indicated that species of Euschongastia in the United States are frequently found only during certain times of the year: i.e., E. rubra Farrell was found only during the winter and spring; that E. luteodema Brennan and E.

maxmotae Farrell were found only in the spring and summer. To further illustrate the seasonal activity of chiggers Brennan and Wharton (1950) point out that in the genus Neotrombicula, "those species that are in the northern group occur on hosts during the summer months only, while those in the southern group are found on hosts during the cooler months" (p. 191).

Rearing

As late as 1944, the complete life cycle of the trombiculid mite was unknown (Ewing, 1944). Early workers trying to study its life cycle found difficulty in rearing the mite past the nymphal stage, primarily due to attempted rearing on improper diets in the post-larval stages. Several Japanese workers (according to Ito et al., 1957) were able to demonstrate with difficulty six major stages of the trombiculid mite, using for the post-larval stages such foods as "sliced potato, various vegetables or fruits and a layer of sweet potato stems." In the United States, Miller (1925) was probably the first individual to record that he reared E. alfreddugesi (?) to "adults." Ewing (1944) postulated that the "adults" of Miller's work were evidently nymphs, as Miller claimed that the nymphal stage was absent. Ewing (1926) reared many nymphs and one adult, but did not observe the prenymphal stage. Even as late as 1944, Ewing, a prominent worker with chiggers, had not seen deposited eggs nor the prenymph stage, and in fact, had doubts that the nymphochrysalis even existed in the North American species of trombiculid mites.

The first breakthrough in the rearing of chiggers came in 1946, when Wharton and Carver (1946), and Jayewickreme and Niles (1946) in separate studies, discovered that the proper food for the post larval stages consisted of eggs and early instar larvae of small arthropods.

Even though the food habits of the post-larval stages were not understood, adults had been reared prior to 1946. Several Japanese workers (according to Wharton and Fuller, 1952) and Mehta (1948) in India had reared chiggers to adults using a "natural" system. As adults were obtained from these methods, it is most likely that small arthropods or their eggs (i.e. collembola or flies) were introduced into the system when grasses or food for the rodents were put into the habitat.

Workers in the United States (Wharton and Fuller, 1952) have relied on using wide-mouthed canning or Mason jars partially filled with a plaster-of-Paris and charcoal mixture for rearing chambers. This type of container can be kept suitably moist, sealed and allows for observation of the developing trombiculid mites. As trombiculid mites are soil-dwelling arthropods a small amount of vermiculite is used for burrowing and for egg laying sites.

In rearing scrub typhus vectors in Malaysia different types of containers were developed (Nadchatram, 1968; Baker $et\ al.$, 1968). Small unglazed clay pots covered with glass sheets were utilized. Moisture was provided by a filter paper wick extending from the bottom of the pot to a pan of water under the pots. A small amount of sterilized soil was used as a substrate for the post-larval stages.

One of the major difficulties in rearing chiggers through to adults is obtaining engorged larvae. Two general methods have been used for obtaining engorged larvae, namely from trapped animals and by feeding unengorged larvae on laboratory animals. The first is simply collecting the larvae from trapped animals. This is usually accomplished by placing the trapped animal over a pan of water and recovering the engorged larvae as they detach from the host and drop into the water. Two variations of placing unengorged larvae on a host have been utilized. Nadchatram (1968) simply placed chiggers in the ear of an anesthesized white mouse and then recovered them from a pan of water after they had engorged. Dohany and Manikumaran (1971) modified this technique slightly by placing a minute drop of water in the ear and then placing the chiggers within this droplet of water. Afterwards a small piece of filter paper was placed over the chiggers, thus absorbing the water and providing a slight pressure on the chiggers. This usually provided for a more rapid and greater percentage of attachment of the chiggers. Baker et~al. (1968) devised a glass capsule that was glued to the shaved back of a mouse. The chiggers were placed on the back within the capsule. The capsule was sealed with a plug of cotton. After engorgement the capsule was removed and the mouse was restrained over a pan of water for recovery of the engorged chiggers.

Farrell and Wharton (1948) devised special vials completely coated with the plaster-of-Paris and charcoal mixture for shipment of engorged specimens from the field. Dohany $et\ al.$ (to be published) shipped engorged larvae of $L.\ (L.)\ akamushi\ and\ L.\ (L.)\ deliense$ (Walch) from Sabah (North Borneo), East Malaysia to Kuala Lumpur,

West Malaysia in screw-capped vials containing only distilled water. These chiggers remained in the water for at least three days with no apparent damage. Shipment of adults of Blankaartia acuscutellaris (Walch) using this same technique also proved successful. In this case, the adults were in shipment for almost two weeks (Dohany, unpublished).

Collecting Techniques

Various collection techniques have been devised for obtaining chiggers. These differ primarily depending upon the intended use of the chiggers. The main collection method for taxonomic studies has relied on the trapping or shooting of animals and removing engorged chiggers. This technique is valuable as it provides a large number of species and provides host data for the chiggers. Live-trapping also provides engorged chiggers that can be utilized for rearing post-larval stages, thus eliminating the difficult feeding step. This is usually accomplished by placing the trapped animal over a pan of water and collecting the engorged chiggers from the water as they detach (Michener, 1946b).

For feeding and biology studies it is often important to collect unengorged chiggers. Most methods are based on the habits of the mite. Probably the most popular current method is the use of a small black board or formica plate, placed on the ground in a suspected chigger site (Gentry, 1965). This board is left in place for several minutes and then picked up and observed for movement of the chigger across the board. The chigger is then removed with a small brush or pointed stick

and placed in a collecting container. Numerous variations of this technique have been devised in recent years (Kundin et al., 1966; Hubert and Baker, 1963a). Crossley and Proctor (1970) compared the collection results of E. splendens and E. alfreddugesi using black plates made of three different materials, "Formica," acrylic plastic and prepainted masonite. The prepainted masonite proved most successful for the collection of these species in Georgia.

Philip (1947) reported that he collected chiggers from the boot tops of troops in the South Pacific. Williams (1946) used a "white-dish" method for his collections. White saucers were used instead of black plates, and the chiggers were collected with an aspirator.

Cockings (1948) and Jones (1950b) employed a light trap method for collection of trombiculid larvae. This was based on the tendency for unengorged chiggers to be positively phototropic. Cockings used a large (3 ${\rm ft}^2$), light, metal pan, while Jones used heavy light-proof paper (2 ${\rm ft}^2$). Both traps had a centrally located tube which allowed the only light to penetrate, thus attracting the chiggers from underneath the trapping area.

Cockings (1948) eliminated the tedious collecting of unengorged chiggers from a pan of water by devising a laboratory light trap. The infested animal was placed in a wooden box which had one side covered with hardware cloth. This open side was attached to a black paper funnel. A filter paper tongue led from the funnel into a petri dish of water. The engorged chiggers were attracted to this dish by a 40 watt bulb placed at the end of the funnel.

Adult trombiculids may be collected by using a floatation technique (Cockings, 1948). Soil is simply placed into a large tub of water and stirred for a short time. The adults can then be collected floating on top of the water. Cockings (1948) indicated that nymphs are not readily collected from this method because they are probably more fragile than the adults and are easily wetted.

Chigger Distribution

Relatively little work has been conducted on the distribution of trombiculid larvae in Florida. Jenkins (1948a) did extensive work on the chiggers affecting man throughout the United States, with a considerable number of his collections occurring in Florida. Ewing (1943), Hyland (1956) and Crossley and Atyeo (1972) have each described one species of chigger from Florida. Several statewide surveys of states other than Florida have contributed vastly to the knowledge of the species and their distribution throughout the United States. Of particular note are the studies of Loomis (1956) of the chiggers of Kansas and Gould (1956) of California. Crossley and Proctor (1971) listed 13 new species records for the state of Georgia

Systemic Insecticide Studies

Previously, methods of control of trombiculid larvae have concentrated on acaricidal sprays, repellants, and cultural control methods. No studies of systemic control of chiggers have been reported in the literature.

Studies of the control of vector chiggers of scrub typhus have been conducted in East Malaysia (Traub et~al.,~1954), West Malaysia

(Traub and Dowling, 1961) and Singapore (Lawley, 1957). Dieldrin (2.5 lbs per acre) applied by either a spray or Swingfog effectively controlled the vectors of scrub typhus for at least 26 months (Traub and Dowling, 1961).

Repellants, such as benzyl benzoate and mixtures of toluamides, have proved effective in controlling chiggers (Gilbert and Gouck, 1953; Gertler et $\alpha\mathcal{I}$., 1962). These repellants, which actually are effective killers of the chigger, are applied by either impregnating clothing or spraying around the ankles, waist and other openings of clothing before an individual goes into a suspected chigger-infested site.

Cultural control methods include clearing of the ground-cover and control of the host animals within a given area (Traub and Wisseman, 1968c). Removal of the ground-cover removes the dwelling places for many of the hosts and allows for drying of the ground, making the habitat unsuitable for all stages of chiggers. Harrison (1956a) concluded that burning grasslands in Malaysia had only a temporary effect in eliminating the habitat of scrub typhus vectors, and that, in fact, it tended to perpetuate the grassland and provides conditions that are actually more favorable to the scrub typhus vector. The removal of the hosts, i.e. rats, will make an area unsuitable for the chiggers. However, as pointed out by Harrison (1956b) and Traub and Wisseman (1968c), the natural hosts must not be replaced by man; otherwise the only thing that would have been accomplished by removal of the host would be a possible increase in the chances of man being infested with the chiggers and thus, in turn, an increased chance of acquiring scrub typhus.

The use of systemic insecticides has proven highly effective against cattle grubs (Drummond, 1968), fleas (Clark and Cole, 1971), lice (Cole and VanNatta, 1967) and mosquitoes (Cole and VanNatta, 1967). Systemic control studies have been conducted on the blood-sucking, northern fowl mite, Ornithonyssus sylviarum (Canestrini and Fanzago). Sevin (N-methyl-1-naphthyl carbamate) applied in the feed of White Leghorn hens provided control for up to 21 days (Kraemer and Furman, 1959). Sulfaquinoxaline (N-[2-quin-oxalinyl] sulfanilamide), a drug commonly used in the control of poultry diseases such as coccidiosis, fowl cholera and fowl typhoid, proved effective, although several weeks of treatment were required before elimination of the mite from the chickens had taken place (Furman and Stratton, 1963). Miller et al. (to be published), while studying field tests of systemics against fleas in New Mexico, observed a decrease in the numbers of "mites" on the rodents tested. A small sample of the mites found on untested animals were identified as Androlaelaps fahrenholzi (Berlese) and Ornithonyssus bacoti (Hirst).

MATERIALS AND METHODS

Collecting Techniques

Chiggers for the distribution studies were primarily collected from litter samples and tree holes, and were obtained by using the Tullgren modification of the Berlese funnel. Some specimens were also collected from black plates. Samples were taken from Tallahassee (Tall Timbers Research Station) in the northern part of the State, from Gainesville in the northcentral part of the State, and a small collection was made from Lakeland in the central part of the State (Figure 1).

Samples obtained either for testing with acaricides or for CO₂ studies were collected solely from black plates. These chiggers were obtained from two main locations: Morningside Park, just east of Gainesville and Gulf Hammock, approximately 40 miles southwest of Gainesville (Figure 1). Chiggers were collected at the Morningside Park location from early summer through October, while chiggers from the Gulf Hammock location were collected in early spring, when they were not available at sites nearer to Gainesville.

Mounting Techniques

Chiggers to be identified were collected in 80 per cent ethyl alcohol and were mounted directly into Hoyer's media on microscope slides (Krantz, 1970). Cover slips were applied and the slides were heated over an alcohol burner until bubbles began to form. After



drying, the cover slips were rung with Glyptal and the cleared chiggers were identified to species. Measurements were made with an ocular micrometer.

Oral Insecticide Application

Insecticides that had shown promise in the control of fleas, lice, ticks and cattle grubs were tested for their systemic activity against chiggers. Techniques used for flea systemic tests were adapted for the chigger tests. Guinea pigs were used as test animals.

For oral insecticide application, the guinea pigs were restrained in a wooden box (Figure 2) measuring 12.70 cm high, 12.00 cm wide and 19.05 cm long. A hole was cut on one end to allow room for the guinea pig's head, and slots were cut in one side to accommodate a small board, thus allowing adjustment for different-sized guinea pigs. A sliding top facilitated placement of the guinea pig within the box.

Figure 3 shows the apparatus used for application of the insecticide. The guinea pig's mouth was held open with a wedge-shaped dowel with holes drilled in it along its length. The wedge-shaped dowel allowed for treatment of guinea pigs having different-sized mouths. A number 10 French Suction catheter (55.88 cm long) attached to a 5 cc hypodermic syringe was passed through the dowel, down the guinea pig's throat and into its stomach. Previous experience showed that when the catheter reached a depth of 7.62 cm, it was into the guinea pig's stomach. Proper placement of the catheter was tested by depressing the plunger of the hypodermic syringe. Pressure against the plunger indicated either a bent tube or placement of the catheter into the lungs. If

Guinea pig restraining box used for oral application of insecticides. Figure 2.



Figure 3. Equipment utilized for administering oral insecticides to guinea pigs.



this happened, the catheter was removed and additional attempts to place the catheter in the stomach were made.

Dimethyl sulfoxide was mixed with the required amount of insecticide to act as a diluent and carrier. The insecticide is then poured into the open syringe and forced into the guinea pig's stomach. The vial holding the insecticide was washed with 0.5 or 1.0 cc of water, depending on the size of the guinea pig. The wash solution was also forced down the tube into the guinea pig's stomach.

Chigger Application

Initially several types of capsules were constructed before a disposable syringe cover-type capsule was adopted. Figure 4 shows four of the types tested. The first was a glass tube which was flanged at one end and cotton used as a stopper (Nadchatram, 1968; Baker et $a\mathcal{I}$., 1968). The small size of the skin area proved unsatisfactory for application of large numbers of chiggers. The upper part of a plastic syringe using the rubber plunger as a stopper worked fairly well, but removal of the rubber stopper was cumbersome. The neck of a screw-cap vial with the screw cap in place was too heavy, and unbroken capsules were difficult to obtain.

After the insecticide was applied, an area on the back of the guinea pig was first clipped with animal clippers and then shaved with a single-edge razor blade. A capsule was constructed by cutting off the expanded end of a disposable 2.5 cc hypodermic syringe container (Figure 5). The capsule was affixed to the guinea pig with Cattle Back Tag Cement, obtained from the Florida State Cattle inspectors.

Figure 4. Four types of capsules used for holding chiggers during their engorgement.



Construction of a chigger-holding capsule from the cover of a disposable $2.5\ {\rm cc}$ hypodermic syringe cover. Figure 5.



A circle of Whatman filter paper was placed in the top of the cap.

This was wetted to provide a high humidity content within the capsule and to allow easy collection of engorged chiggers from water droplets that formed in the cap.

Two hours after the insecticide was administered, 100 chiggers were applied to the back of the guinea pig inside the capsule. The chiggers were placed in a minute droplet of water (Dohany and Manikumaran, 1971). This drop of water prevented movement of the chiggers until all the chiggers could be applied. After all the chiggers were in the droplet of water, a small piece of Whatman filter paper was placed on top of the chiggers. This piece of paper absorbed the water and facilitated attachment of the chiggers.

Feeding Tests

When an insecticide was found to be effective, bait tests, using insecticide-treated food, were attempted. Guinea pig chow was treated by mixing the required amount of insecticide with sufficient quantitities of acetone to cover the feed being treated. The acetone was allowed to evaporate at room temperature, usually for about eight hours. The insecticide-acetone feed mixture was stirred occasionally to allow proper treatment.

Recovery of Chiggers

After the chiggers were applied, a collar was fitted on the guinea pig to prevent the guinea pig from chewing off the capsule (Figure 6). The collar was made from a plastic-coated, rubber-backed placemat and was oval in shape to allow for normal movement of the guinea pig.





The guinea pigs were held in small animal cages for 2 days (Figure 7). At the end of this period, the number of engorged chiggers was counted and recorded. The capsules were then examined every 4 hours and engorged, detached chiggers were collected for rearing to the post-larval stages. The engorged chiggers and post-larval stages were held in 30 cc plastic containers. A circle of Millipore filter paper was cut to fit the bottom. This filter pad was kept moist by the addition of a few drops of water at regular intervals. The entire container was held in a humidity chamber at 100 per cent relative humidity.

CO₂ Measuring Equipment

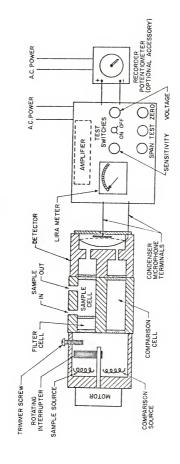
Carbon dioxide production from chiggers was measured on a M-S-A Model 200 LIRA analyzer. This analyzer is a Luft Type Infrared Analyzer which measures the concentration of one component of a mixture of gas. This measurement is accomplished by measuring the infrared absorption of a desired component within the mixture.

Two similar helices of nicrome wire (Figure 8) are heated to a dull red color (1200°F). Infrared radiation emitted from these helices is channeled through two separate, parallel cells to a detector. The detector transforms infrared energy into an electrical signal which can be amplified and read on the LIRA meter or from a recorder.

The two cells utilized by the analyzer are 1) a comparison cell and 2) a sample cell. Prior to testing, the analyzer is "zeroed" by introducing the same gas to each cell, in this case, normal air. To begin the test, gas from the sample containing chiggers (normal air

Figure 7. Guinea pig holding cages used in systemic acaricide tests against chiggers.





Schematic diagram of a M-S-A Model 200 LIRA infrared analyzer. Figure 8.

OPTICAL BENCH

ELECTRICAL CONTROLS

¹kxtracted from installation and operation manual; Technical Products Division; Mine Safety Appliances Company; 201 N. Braddock Avenue; Pittsburg, Pennsylvania 15208.

plus added ${\rm CO}_2$ produced by the chiggers) is directed through the sample cell, while normal air is continued through the comparison cell. Some of the infrared energy that is being channeled through the sample cell is absorbed and thus the amount of radiation reaching the detector is reduced.

Built into the analyzer is a rotating interrupter blade, which causes only one of the two beams of radiation to strike the detector at any one time. As the radiation from the beams strikes the detector, a detector gas is heated and expansion occurs. When the two gases within the cells are the same, equal expansion occurs and the "zeroing" effect is obtained. When a different gas is introduced into the sample cell, an unequal amount of radiation enters the detector causing the expansion of the detector gases to be unequal also. Thus, the detector directly measures the difference between the two expanded gases. This reading is obtained by a microphone membrane that moves in response to the pressure changes of the detector gas. An electrical signal is generated which is proportional to the difference between the two radiation beams. An Esterline Angus, Speed Servo II (Model L1101S) strip-chart recorder was used for recording the output of CO2 from the chiggers.

Constant Temperature Chamber

A plywood, insulated box, measuring 39.37 cm wide by 38.10 cm high by 77.47 cm long, was utilized as a constant temperature chamber

¹Esterline Angus, Division of Esterline Corp., Box 24000, Indianapolis, Indiana 46224.

(Figure 9). This box was divided into a testing area and a heating area. The heating of the chamber was accomplished by using two incandescent lamps at the rear of the box. A fan was located between the two divisions of the chamber to circulate air within the entire chamber. A regulating thermostat was located in the testing area to operate the heating elements.

Chigger Testing Chamber

A chigger testing chamber was devised by modifying a Nalgene Millipore Filter Unit (Figure 10). The millipore filter was removed from the bottom of the upper chamber, leaving the fiber pad in place for use as a substrate by the chiggers. The chiggers could not move through this filter pad, but air could easily pass through it. The sides of the chamber were coated with a 1:9 charcoal-plaster-of-Paris mixture.

An inlet port was affixed to the top of the chamber. Holes were drilled into the top for placement of humidity and temperature sensors. Rubber corks with the sensors placed in them were used to close those holes, thus preventing escape of air, CO_2 and chiggers. A thin rubber gasket was placed between the top and the chamber proper, providing a tight seal. The entire chamber was sandwiched together between two pieces of plexiglass with four six-inch screws.

¹Nalgene Labware Division, Nalge Sybron Corp., Rochester, N. Y. 14602. Catalog No. 245.

Figure 9. Constant temperature chamber used for carbon dioxide studies.



Left, millipore filter chamber before modification. Right, modification of millipore filter chamber used for holding chiggers during carbon dioxide studies. Figure 10.

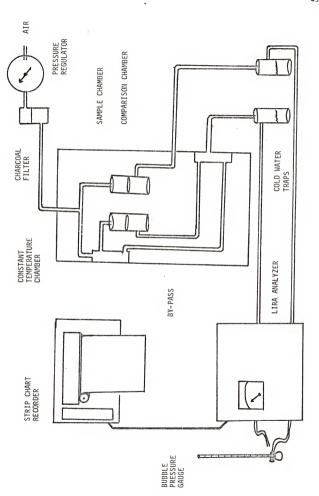


General Operation of the CO₂ Analyzer

Figure 11 shows a schematic diagram of the layout of the constant temperature cabinet, LIRA analyzer and recorder, while Figure 12 is a photograph of the actual equipment as it was set up for operation.

Normal laboratory air, having a composition of approximately 300° ppm CO_2 , was utilized for operation of the LIRA analyzer. A pressure regulator set at 3 psi was used to maintain a constant input of air. The air was first passed through a charcoal filter to remove its impurities, and then was taken into the box. Tygon tubing was utilized throughout the entire system for transporting the air. Within the constant temperature chamber the air was divided and sent through two lines: a sample and a comparison line. The sample air could then be passed into the sample or into a bypass line. The bypass line allowed for "zeroing" the equipment and allowed for buildup of CO_2 within the sample container for CO_2 accumulation tests.

After leaving the constant temperature chamber, the air from both the sample and comparison lines was passed through cold water traps to remove the moisture from the air. Sensitive, screw-type regulators were placed in the air lines to adjust the flow of the air throughout the entire system. A bubble pressure gauge, constructed from a 25 mm burette, was used to measure the flow rate of the air. The flow rate was measured from the outlet ports of the LIRA analyzer and under normal operations was set at 60 cc per minute.



Schematic diagram of equipment used for carbon dioxide studies. Figure 11.

Equipment used for carbon dioxide studies, showing constant temperature chamber, LIRA analyzer, and strip-chart recorder.

Figure 12.



Temperature and Humidity Measuring Instruments

A Digitec digital thermometer system was used to monitor the temperature in both the constant temperature cabinet and the chigger testing chamber. A banjo-type thermister probe was held in place by a rubber stopper in the top of the chigger testing chamber.

A Dunmore-type electrical hygrometer (Dunmore, 1938) was constructed to measure the humidity within the chigger testing chamber (Figure 13). The basis of a Dunmore hygrometer is that a hygroscopic salt bridge is made between two electrodes. An electrical current is passed between the two electrodes. As the salt bridge absorbs or looses moisture to the atmosphere, the electrical resistance of the bridge also changes. This change in resistance is then measured and a direct reading of the humidity can be obtained.

The sensory element was constructed by inserting two small pieces of stainless steel wire into two capillary tubes. The tips of the stainless steel wire were allowed to extend a fraction of an inch past the end of the capillary tubes. The ends of the capillary tubes with the small amount of protruding wire were heated with a propane torch until they melted slightly and rounded off. The two capillary tubes are placed side by side with the two sensory wires adjacent to but separate from each other. The capillary tubes were affixed to each other with silicon adhessive. The nonsensory end of the stainless steel wires was soldered to copper wires which were connected to the indicating instrument. The sensory tip was coated

Digitec, Model 501. United Systems Corp., Dayton, Ohio.

Dunmore-type electrical hygrometer used for measuring humidity within the chigger-holding container. Figure 13.



with a lithium chloride solution, as prescribed by Rogers (1957) before it was put into operation. Each sensory element was calibrated with known salt solutions prior to its use.

The indicating instrument was constructed along the general plans set forth by Rogers (1957).

RESULTS

Chigger Identification Studies

Surprisingly little work has been conducted on the chiggers found in the State of Florida. Thus, before the systemic insecticide studies were begun chiggers were collected for identification of the primary chiggers present within the study area. Although relatively few samples were taken, six species were found that were new to the State of Florida, and two are recorded from their hosts for the first time

Eutrombicula

The most common chiggers that attack humans in the United States are in the genus Eutrombicula. The two most frequently occurring in the southeastern United States are E. splendens (Ewing) and E. alfreddugesi (Oudemans). Jenkins (1948a) presented the host and habitat information on these two species in detail. Numerous specimens of both species were collected from all locations, primarily using the black plate collection technique and from live trapped rodents. These species can be differentiated taxonomically by the number of their dorsal setae: E. alfreddugesi having 24 to 28, while E. splendens has 22 (Wolfenbarger, 1952).

The genus Eutrombicula was originally proposed by Ewing in 1938 with the type species being Microthrombidium alfreddugesi Oudemans.

Since the original proposal, Eutrombicula has oscillated back and forth between subgeneric and generic status. It is currently held by taxo-

nomists to be of generic ranking. Nadchatram and Dohany (1974) present the following diagnosis of the genus:

PIF 7BS. GAL N. CL 2 pronged, axial or external prong usually longer than accessory or internal prong. Chelicerae long usually with a small dorsal and ventral apical tooth. Eyes 2 + 2. Scutum subquadrate or quadrate, never pentagonal. Sensillae slender with distal barbs. Legs 7-7-7 segmented. 2 or 3 genualae I. A long outstanding mastitarsala III always present and mastitibiala III occasionally present. (p. 55)

No new collection records were made during this study. Jenkins (1948a), Wharton and Fuller (1952) and Wolfenbarger (1952) present an abundant number of hosts from which each of these species have been collected. Table 3 presents the scutal measurements 1 of both E . alfreddugest and E . splendens collected during this study, as well as a compilation of the measurements made by previous workers who have studied these species.

Fonsecia

Two species of the genus Fonsecia were collected: one of the subgenus Fonsecia and the other of the subgenus Farasecia. The genus Fonsecia is characterized by having the PL's greater than the AM and the AM greater than the AL's. The subgenus Farasecia differs from the subgenus Fonsecia by having normal AL scutal setae, while they are peg-like in the subgenus Fonsecia. Radford (1942) originally proposed the raising of two Brazilian species to the generic level and designating them Fonsecia as the "antero-lateral setae were represented by tooth-like projections." Brennan and Loomis (1959) in their review of

Much of the taxonomic differentiation of genera, subgenera and species of chiggers is based on the size, shape and conformation of the dorsal shield or scutum. Figure 14 presents a diagram showing the structures or parts of the scutum that are measured.

Figure 14. Diagram of a chigger scutum showing structures and standard scutal measurements.

Abbreviations:

AW = distance between anteriolateral setae

PW = distance between posteriolateral setae

SB = distance between sensillae bases

ASB = distance from anterior margin to sensillae bases

PSB = distance from posterior margin to sensillae bases

A-P = distance between anteriolateral and posteriolateral

AM = length of anterior median seta

AL = length of anteriolateral setae

PL = length of posteriolateral setae

S = length of sensillae

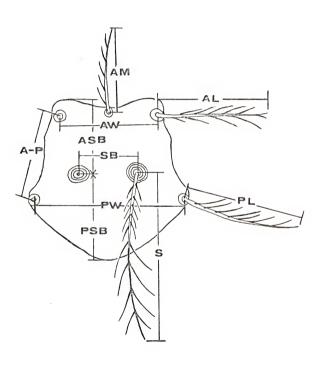


Table 3. Scutal measurements of Eutrombicula alfreddugesi (Oudemans) and E. splendens (Ewing), and a comparison of measurements made by previous chigger taxonomists.

	AW	PW	SB	ASB	PSB	A-P	AM	AL	PL	S
Eutrombicula alfreddugesi	-									
Florida A B C D E F G	72 77 87 78 80 81 88	87 90 94 93 92 93 94	40 42 45 45 47 46 44	22 22 24 24 25 24 25	31 25 30 30 27 31 30	27 25 25 27 26 26 27	31 37 30 36 27 30 40	29 32 36 33 30 31 32	42 47 49 46 44 47 49	45 51 47 49 44 53
Average Jenkins (1949b) Wolfenbarger (1952)	81 81	92 90	44 43	24 23	29 31	26 27	33 33	32 33	46 47	48 50
Kansas	77	88	43	23	26	27	28	29	40	49
Eutrombicula splendens										
Florida A B C D E F G	80 82 84 85 80 87 78	92 95 95 95 92 100 91	43 45 45 47 43 49	23 26 27 26 25 24 22	28 28 30 32 30 30 30	25 26 28 28 29 29	38 41 40 40 37	34 33 38 35 36 23	50 48 50 50 52 48 48	 51
Average Jenkins (1949b)	82 78	94 91	45 44	25 22	30 32	27 27	39 37	35 34	49 48	51 51
Wolfenbarger (1952) Missouri Arkansas Texas	83 92 100	94 104 112	43 47 50	24 25 25	28 31 31	27 30 29	33 35 36	34 33 33	47 47 47	52 53 52

the genus, placed seven species in *Fonsecia*. Loomis (1956) established the subgenus *Parasecia* with *F.* (*Parasecia*) *gurneyi* (Ewing) being the type species and referring six additional species to the subgenus.

The diagnosis of *Fonsecia*, as established by Brennan and Loomis (1959) is:

Trombiculine larvae having a scutum with stubby, peg-like anterolateral setae; branched, flagelliform sensillae; anterior setae set back from margin. Cheliceral blade with tricuspid cap, dorsal and ventral tooth; galeal seta long, nude. Palpal genual and laterotibial seta nude; palpal tarsus with 6 branched setae, a subterminala and a tarsala Legs with 3 genualae I, tarsala I longer than tarsala II, no mastisetae III; only 1 branched seta on each coxa. (P. 53)

The diagnosis of the subgenus *Parasecia* as described by Loomis (1966) is:

Similar to the subgenus Fonsecia Radford, 1942, in having scutum with anterior setae set back from margin; ALSAMSPL; sensilla flagelliform; galeal seta nude; palpal tarsus with 7B.S.; 2 or 3 prongs on palpotibial claw; usually 3 genualae I; differing from subgenus Fonsecia in having normal AL scutal setae (peglike in Fonsecia). (p. 191)

Brennan (1969) in adding three new species to the subgenus

Parasecia stated that; "Parasecia is further distinguished from

Fonsecia, whose four species are also restricted to the New World,

by a sinuous posterior scutal margin (convex in Fonsecia) and a wide

host range (Fonsecia species apparently confined to reptiles)" (p. 662).

Fonsecia (Fonsecia) palmella Brennan and Loomis was collected from Berlese samples of five treeholes and a sample of Spanish moss (Tillandisis usneoides) from the Tallahassee area. This species had been previously collected from the southeastern five-lined skink

 $^{^{\}rm l}$ Three of the original seven species of $\it Fonsecia$ were placed in the new genus $\it Fonsecula$ (Loomis, 1966).

(Eumeoes inexpectatus) and from the five-limedskink (E. fasciatus) from Louisiana. This is the only species of Fonsecia (Fonsecia) that has been reported from the United States. Table 4 presents the scutal measurements of five specimens of F. (F.) palmella. Figure 15 shows the scutum of F. (F.) palmella.

Table 4. Scutal measurements of Fonsecia (Fonsecia) palmella Brennan and Jones.

B 55 69 26 27 17 13 41 12 38 6 C 54 70 26 27 16 13 46 12 40 66 D 56 69 26 25 17 12 41 11 35 6 E 55 71 27 28 17 12 42 11 39 66 Average, 55 70 26 27 17 13 43 12 38 66	Slide	AW	PW	SB	ABS	PSB	A-P	AM	AL	PL	S
B. & L. 1 57 71 27 30 17 14 42 12 37 63	C D E Average,	55 54 56 55	69 70 69 71	26 26 26 27 26	27 27 25 28 27	17	13 13 12 12 13	41 46 41 42 43	12 12 11 11 12	38 40 35 39 38	69 64 63 61 62 64 63

Fonsecia (Parasecia) gurneyi gurneyi (Ewing) was the most abundant chigger collected from Berlese samples of treeholes. This species was collected from all the collection sites in Gainesville, Tallahassee and Lakeland. This species has been reported throughout the southeastern United States, as far north as Maryland and as far west as Texas. Loomis (1956) lists a large number of reptiles and mammals that F. (P.) gurneyi gurneyi was collected from and determined that its principal host in eastern Kansas seemed to be the five-lined skink (E. fasciatus). Table 5 presents the scutal measurements of 10 specimens of F. (P.) gurneyi gurneyi. Figure 16 presents a photomicrograph of the scutum of F. (P.) gurneyi gurneyi gurneyi.

¹Brennan and Loomis (1959).

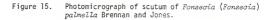


Figure 16. Photomicrograph of scutum of Fonsecia (Parasecia) gurneyi gurneyi (Ewing).





Table 5.	Scutal measurements of Fonsecia	(Parasecia)
	gurneyi gurneyi (Ewing).	

AW	PW	SB	ASB	PSB	A-P	AM	AL	PL	S
65	76	29	25	15	17	31	26	45	61
64	75	32	24	16					
64	74	32	24	17	16				54
67	79	32	25	17	17	27	26		54
66	76	31	21	17	16	31	25		53
62	74	32	23	16	17	30	24	40	
64	76	30	31	18	17	31	24	40	55
65	78	29	22	17	18	31	24	41	56
66	76	31	24	16	18	32	25		53
65	76	31	24	16	18	30	27		59
65	76	31	23	16	17	30	25		56
61	72	28	22	16	15	28	23	41	48
	65 64 64 67 66 62 64 65 66 65	65 76 64 74 67 79 66 76 62 74 64 76 65 78 66 76 65 76	65 76 29 64 75 32 64 74 32 67 79 32 66 76 31 62 74 32 64 76 30 65 78 29 66 76 31 65 76 31	65 76 29 25 64 75 32 24 64 74 32 24 67 79 32 25 66 76 31 21 62 74 32 23 64 76 30 31 65 78 29 22 66 76 31 24 65 76 31 24	65 76 29 25 15 64 75 32 24 16 64 77 32 24 17 67 79 32 25 17 66 76 31 21 17 62 74 32 23 16 64 76 30 31 18 65 78 29 22 17 66 76 31 24 16 65 76 31 24 16 65 76 31 24 16	65 76 29 25 15 17 64 75 32 24 16 18 64 79 32 25 17 17 66 76 31 21 17 16 62 74 32 23 16 17 64 76 30 31 18 17 65 78 29 22 17 18 66 76 31 24 16 18 65 76 31 24 16 18 65 76 31 24 16 18	65 76 29 25 15 17 31 64 75 32 24 16 18 30 64 74 32 24 17 16 26 67 79 32 25 17 17 27 66 76 31 21 17 16 31 62 74 32 23 16 17 30 64 76 30 31 18 17 31 65 78 29 22 17 18 31 66 76 31 24 16 18 32 65 76 31 24 16 18 32 65 76 31 23 16 17 30	65 76 29 25 15 17 31 26 64 75 32 24 16 18 30 24 64 74 32 24 17 16 26 23 67 79 32 25 17 17 27 26 66 76 31 21 17 16 31 25 62 74 32 23 16 17 30 24 64 76 30 31 18 17 31 24 65 78 29 22 17 18 31 24 66 76 31 24 16 18 32 25 65 76 31 24 16 18 30 27 65 76 31 24 16 18 30 27 65 76 31 24 16 18 30 27 65 76 31 24 16 18 30 25	65 76 29 25 15 17 31 26 45 64 75 32 24 16 18 30 24 42 64 77 79 32 25 17 17 27 26 41 66 76 31 21 17 16 31 25 44 62 74 32 23 16 17 30 24 40 64 76 30 31 18 17 31 24 40 64 76 30 31 24 16 18 32 25 43 65 76 31 24 16 18 32 25 43 65 76 31 24 16 18 30 27 45 65 76 31 23 16 17 30 25 42

Euschongastia

Two species of Euschongastia were collected during this study.

Farrell (1956) conducted a detailed study of the Euschongastia of North
America. Farrell (1956) (after Fuller, 1952) presented the following
diagnosis of the genus:

All legs with seven segments; true stigmata and tracheae absent; empodium clawlike; no caudal plate; eyes with five setae in addition to sensillae; scutum not submerged beneath the cuticular striae; sensillae expanded distally; chelicerae bladelike, each with a single dorsal tooth; palpal claw with two to seven prongs. (p. 124)

Euschongastia peromysci (Ewing) was taken from Peromyscus floridanus from the Gainesville area. This species has been previously reported throughout the northeastern United States as far west as Oklahoma. Crossley and Proctor (1971) reported this species from the eastern chipmunk (Tamias striatus) and the pine vole (Microtus pinetorum) from Georgia. This is the first record of this species from Florida.

Euschongastia peromysci can be easily distinguished by its unusual galeal setae, having 1-3 stiff setules that arise near the base and by its characteristic scutal shape. Figure 17 is a photomicrograph of these characteristics and Table 6 presents the scutal measurements of five specimens.

Table 6.	Scutal measurements	of	Euschongastia
	peromysci (Ewing).		3

Slide	AW	PW	SB	ASB	PSD	A-P	AM	AL	PL	S
A B C D E E Average Farrell1	50 48 51 49 50 50	59 57 58 58 57 58	15 14 14 14 14 22	31 39 30 29 30 30 27	13 14 13 15 11 14 8	18 17 17 15 16 17	36 37 34 34 34 35 27	53 54 55 50 56 54 35	55 53 57 53 59 55 46	24 X 15 21 X 15 23 X 15 X 16 25 X 15 23 X 15 27 X

A single specimen of <code>Eusohongastia setosa</code> (Ewing) was collected from a treehole in Gainesville in December, 1971. Additional specimens were not collected, even though extensive samples were made from this same treehole throughout the year. This collection in the winter corresponds with previous collections of this species. Farrell (1956) records this species occurring from late fall through spring in North Carolina and Pennsylvania. The specimen of <code>E. setosa</code> (a co-type) that was previously collected from the closest locality to Gainesville, Okefenokee Swamp, Georgia, was also collected during December.

 ${\it Euschongastia~setosa} \ \ {\it is~characterized~by~its~scutum~being~wider}$ than the PL and the sensillae heads being ovoid. Figure 18 shows

¹Farrell (1956). Average of 100 specimens.

Figure 17. Photomicrograph of scutum of Euschongastia peromysci (Ewing).

Figure 18. Photomicrograph of scutum of Euschongastia setosa (Ewing).





the scutum. The scutal measurements for the one specimen collected are:

<u>Microtrombicula</u>

Microtrombicula crossleyi (Loomis) was collected only from a treehole from the Tallahassee area. This species has previously been reported from Kansas and Oklahoma (Loomis, 1954).

Vercammen-Grandjean (1956) in his revision of the genus Microtrombicula presents the following diagnosis:

Trombiculini having a pentagonal shaped scutum with anterior "shoulders" and backwarded anterolateral setae; its sensillae either branched or flagelliform; two-pronged; always oculate and provided with one mastitarsala 3 and a single genuala 3. (p. 37)

Microtrombicula crossleyi is closely related taxonomically to M. trisetica (Loomis and Crossley) both having three pairs of sternal setae and multiple coxal setae. The chief characteristics separating the two species are the reduced scutum of M. trisetica with three setae on coxa III while M. crossleyi has five setae on coxa III. Figure 19 shows the general scutal shape, while Table 7 lists the scutal measurements of M. crossleyi.

Walchia

Walahia americana Ewing was collected from a treehole in Gainesville.

This species was originally described by Ewing (1942) from a "cotton mouse" (Peromyscus gossypinus [?]) from Tallahassee. It has been collected from numerous mammal hosts. Loomis (1956) indicates that

this species, like all of the members of the subfamily Walchiinae, seems to occur only upon mammals. In addition to the type location, this species has been collected from Wisconsin (Farner, 1946), Oklahoma, Nebraska, Iowa, Kansas, Utah (Loomis, 1956), California (Gould, 1956) and Georgia (Crossley and Proctor, 1971).

Table 7. Scutal measurements of Microtrombicula crossleyi (Loomis).

Slide	AW	PW	SB	ASB	PSB	A-P	AM	AL	PL	S
A B C D E Average V-G	34 36 33 32 32 32 33 35	42 42 41 41 37 41 46	13 13 11 13 12 12 12	22 20 21 21 20 21 22	23 21 19 19 21 21 20	20 21 19 21 22 21 22	19 16 14 18 17 17	18 16 18 17 16 17	25 24 22 22 24 23 57	32 29 25 28 30 29 34

The scutum of *W. americana*, as with the entire genus, has only four scutal setae: 2 AL's, 2 PL's but lacking an AM. Figure 20 shows the unusual scutum of *W. americana* and Table 8 presents the scutal measurements.

Table 8. Scutal measurements of Walchia americana Ewing.

Slide	AW	PW	SB	ASB	PSB	A-P	AM	AL	PL	S
A	47	70	41	20	48	29		25	27	50 X 10
B	45	70	40	19	47	29		28	28	50 X 10
Average	46	70	40	19	47	29		26	27	50 X 10
Loomis	45	68	38	20	44	30		25	27	51 X

¹Loomis (1956). Average of 7 specimens.

Figure 19. Photomicrograph of scutum of *Microtrombicula crossleyi* (Loomis).

Figure 20. Photomicrograph of scutum of Walchia americana Ewing.





Insecticide Screening Tests

Insecticides used for the screening tests were chosen from insecticides that had previously proved to be effective systemic insecticides for control of lice, fleas or other ectoparasites, or were known to be good repellants or acaricides. Usually the highest dosage that was not lethal to the guinea pig was used. Such information was obtained from previous studies conducted by the Louse and Flea Sections of the United States Department of Agriculture, Insects Affecting Man Laboratory and from other previous systemic studies. The insecticides studied, as listed in Table 9, were selected from eight different chemical or use groups as categorized by the Entomological Society of America (Kenaga and Allison, 1969; 1970). Complete nomenclatures, chemical and toxicological descriptions, as presented by Kenaga and Allison (1969; 1970), can be found in Appendix A.

Two slightly different treatments were conducted in the screening tests. In the early tests, the insecticides were administered two hours before the application of the chiggers. Table 10 presents the data for the early tests. Many of the insecticides used were long residual insecticides. In the later tests, in which much of the insecticides were short-lived organophosphates, the chiggers were applied shortly before the administration of the insecticides (Table 11).

As rearing of engorged chiggers to post-larval stages did not prove successful, the effectiveness of an insecticide was based on attachment and engorgement of the chigger. Tables 10 and 11 summarize the recoveries of engorged chiggers from guinea pigs treated with the

Table 9. Insecticides used for screening experiments, as listed by chemical or use groups.

Group and Common Name ²	(ENT) Number
Chlorinated Aryl Hydrocarbons C 2 lindane C 8 endosulfan C 12 mirex	7796 23979 25719
Miscellaneous Compounds M 2 Morestan M 7 GALECRON M 9 Tarzol	25606 27335 27438
Aliphatic Derivatives of Phosphorous Compounds PA 20 dimethoate	24650
Aryl (Phenyl) Derivatives of Phosphorus Compounds PC 20 PROBAN PC 21 famphur PC 38 phoxim	25640 65644 27448
Heterocylic Derivatives of Phosphorous Compounds PH 13 RAMETHIN PH 16 Dursban PH 18 diazinon	25567 27311 19507
Repellants R 5 benzyl benzoate	
Sulfites S 9 Omite	27226
Carbamates X 9 MESUROL X 15 carbaryl	25726 23969

 $^{^1\}mathrm{As}$ listed by Kenaga and Allison (1969) Bull. Entomol. Soc. Am. 15: 85-148; Revised in accordance with changes in Bull. Entomol. Soc. Am. 16: 68.

 $^{^2\}mathrm{Common}$ names listed are as required for use in publications of the Entomological Society of America. Chemical names or trademarks are listed when a common name was not available from the above listed publication.

Summary of early insecticides administered orally to guinea pigs during systemic screening tests against chiggers, showing the number of chiggers recovered during 12-hour intervals. Table 10.

Insecticides	Dose (mg/kg)	Number Engorging	0-35 36-47 48-59 60-71 72-83 84-95 96-1107 108-119 132-131 132-143 144-155 166-167	Recovered	Per Cent Recovered
		34	000000040000-0	20	58.8
2.0		10	000-0-000000	4	40.0
Galecron	45	17	000040-000	10	58.8
		36	0 - 2 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	56	72.2
		22	00000000-040	21	95.5
Dursban	25	38	0004900106122	28	72.7
[nzuog		23	000000000000000000000000000000000000000	22	95.7
Benzoate Benzoate	200	30	0 % 6 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	13	43.3
		25	0-0-00-88-600	15	0.09
Pusbnil	5	=	00-00000	9	54.5
		16	00-0%-900-000	12	75.0
		22	000071752000	19	86.4
Lindane	-	25	000000000000000000000000000000000000000	12	48.0
		25	0	Ξ	44.0

Table 10. Continued.

Norma 1		40	00084480001	52.5
[caso)		28	00 2 4 5 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0 0	100.0 52.5
25 BF 71 Shell	100	20	000076871000000	0.00
She11	-	22	000000000	50.0 100.0
nonizsiO	200	30	00001000474900	198.7
	2	29	01081401008	89.6 86.7
UB2 C2 10U	200	23	000 000 000 000 000 000 000 000 000 00	0.00
Morestan	25	31	00088808788100 45	77.4 100.0
Endosulfan	20	27	000000000000000000000000000000000000000	85.2
ucy[sop=]	2	27	0-00%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%%	88.9
xəniM	300	28	01008884448000	0.00
n	36	9	000000000000000000000000000000000000000	100.0 100.0
nixodq	300	40	2 0 0 0 0 0 0 0 0 0 1 1 1 1 1 1 1 1 1 1	42.5
-		27	10 00 00 00 00 00	37.0 42.5
nivə2	100	20	000000000000000000000000000000000000000	40.0
Insecticide	Dose (mg/kg)	Number Engorging	0-35 36-47 48-59 48-59 60-71 72-83 84-95 96-107 108-119 132-443 144-155 156-167 168+	Per Cent Recovered

Table 11. Summary of later insecticides administered orally to guinea pigs during systems creening tests aginst chiggers, showing the number of chiggers recovered during 12-hour intervals.

	Insecticide Mensurol	Dose (mg/kg) 25	Number Engorging 28	0-47	18-59 0	72-83	34-95 4	16-107 4	38-119		32-143 3				Total Recovered 16	Per Cent	
	əfimO	009	33	0	0 0	4 C	~	2 1	7	0	က	0	C	0	16	u a	40.0
	пiјэmвЯ	75	35	0	<u>-</u> ч	n er		7	7	2	0	0	C	0	35	0	0.00
, c , a , a , a , a , a , a , a , a , a	Proban	20	28	0	m n	. 6	1 4	0	0	0	0	0	· C	0	22	2 02	18.0
	mixodqyoldƏ	1,000	57	0	~ 0	00	o (*	· –	و.	ω	_	00	-	- 2	32	. 25	1.00
	[ozns]	20	26	-	00		1 (۰ د	ו עמ	9	c	۰ ۸	10	0	24	ć	92.3
	Famophos	200	39	-	m	ט ר	٠ ١	n er	m	00	C	0	0 0	00	25	,	1.49
	Galecron	50	26	m	2 0	V C) F	- 4	o (*	0 0	· C) C	0 0	00	17	L	65.4
	Dimethoate	150	0	0	00	0	0 0	0 0	0 0	0 0	· C	0	0 <	0	0		1

various insecticides at twelve-hour intervals. Differences between the number of chiggers engorging and the total number of chiggers recovered were caused by desication within the capsule. Although moist filter paper was placed in the top of the capsule, often the chigger would not be able to reach this moist area and died. Death was often caused by the chigger becoming stuck in the cement used to affix the capsule to the guinea pig or by becoming stuck to the hairs of the guinea pig or sides of the capsule by the fecal material extruding from the engorged chigger.

During the screening tests, 1,004 chiggers were observed engorging, representing approximately 35 per cent engorgement. The per cent of chiggers recovered ranged from 40.0 to 100.0 per cent, with an overall average of 64.9 per cent.

Of the insecticides listed, only dimethoate at 150 mg/kg caused complete kill of the chiggers. On first observation, after 24 hours, all the chiggers attached to the quinea pig were dead.

Oral Applications of Dimethoate to Guinea Pigs

This success of dimethoate (ENT 24650) at 150 mg/kg demonstrated that an insecticide could be utilized for the control of chiggers, thus, proving that an insecticide or acaricide could function as a systemic for chigger control. In order to obtain information that could be used for application of dimethoate to actual host animals, additional tests were conducted. Such tests were important to determine what levels of the acaricide were necessary for complete control. The original intent was to determine the LD/50 of this acaricide to chiggers.

Dimethoate is a general usage insecticide distributed under the trade name CYGON (Trademark of the American Cyanamid Company).

Dimethoate is recommended for usage in agriculture insect control, housefly control, forest insect control, ornamental insect control and as a plant systemic for the control of insects on certain fruits (1973 Pesticide Product Guide; American Cyanamid Company; Princeton, New Jersey 08540). The chemical name of dimethoate is 0,0-dimethyl S-(N-methylcarbamoylmethyl) phosphorodithioate and is represented by the structural formula:

Several previous studies utilizing dimethoate as an animal systemic insecticide have been made with various insects. Of eleven related compounds, Hewitt et~al. (1958a) found that in studies against Aedes aegypti (L.) dimethoate was the most active and, with only one exception, had the widest margin of safety between aedicidal doses and doses toxic to mice. They also found that single oral doses of 12.5 mg/kg dimethoate effectively killed 50 to 80 per cent of lice (Polyplax sp.) which were naturally infesting the mice.

Drummond (1958) found that dimethoate, in tests in which guinea pigs were used as hosts, would give 100 per cent kill at a level of 10 mg/kg for screwworms (Callitroga hominivorax [Cqrl.]) and stable flies (Stomoxys calcitrans [L.]) and at a level of 25 mg/kg would kill 100 per cent of tested Lone Star ticks (Amblyomma americanum [L.]). In tests with sheep and goats, dimethoate was effective at 50 mg/kg

for both nymphs and adults of the Lone Star tick and as low as 25 mg/kg for screwworms and stable flies.

Hewitt et al. (1958b) found that single oral doses of 5 mg/kg or more of dimethoate administered to Hereford calves produced 100 per cent kill within one week to the second instar of the cattle grub, Bypoderma lineatum (De Vill.), while similar tests on another cattle grub (H. bovis [L.]) killed 90 to 100 per cent. Their tests showed that doses lower than 10 mg/kg were not as effective against third instar larvae, but at 10 mg/kg and above the lethal rates were similar. Single oral or intramuscularly administered doses of dimethoate were much less effective on first instar larvae of H. Lineatum.

Cole and VanNatta (1964), in a study of twelve organophosphorus compounds, found that dimethoate at 25 mg/kg given orally to rabbits produced 60 per cent kill to the human body louse (*Pedicalus humanus humanus L.) one hour after treatment. No mortality was noted after this time. With 50 mg/kg doses, lice were killed at one, two and three hours after treatment, with a mortality rate of 100, 100 and 94 per cent respectively. Louse kill did not occur after the third hour.

Kaplanis et al. (1959) studied the metabolism of 32 P-labeled dimethoate in cattle. The labeled dimethoate was administered both orally and intramuscularly. Radioactivity was noted in the blood shortly after administration. Approximately 87 to 90 per cent of the dimethoate given to the calves was accounted for in the urine 24 hours after oral treatments and 9 hours after the intramuscular treatments. Only very small amounts of insecticide were found to be eliminated in the feces.

Table 12 presents the data of the oral tests that were administered to guinea pigs for the control of chiggers. The dimethoate doses for these tests ranged from 25 to 150 mg/kg.

Table 12. Systemic tests of dimethoate administered orally to guinea pigs for the control of chiggers.

Dose (mg/kg)	Number Engorging	Number Recovered	Per cent Mortality
25	48 53	42 40	0
50	60 26	54 24	0
75	2 1		96 99
90	0	0	100
. 100	0	0	100 100
150	0	0	100

In the 25 and 50 mg/kg tests the chiggers became fully engorged and a high percentage of the engorged chiggers were recovered. With the 75 to 150 mg/kg tests, virtually 100 per cent of the chiggers were killed. Only in the 75 mg/kg tests did any chiggers become engorged, with 2 of 57 (96 per cent mortality) in one test and 1 of 78 (99 per cent mortality) in the second test.

Feeding Experiments of Dimethoate to Guinea Pigs

After successfully controlling chiggers through oral doses of dimethoate administered to guinea pigs, the next important step was

to determine if chiggers could be killed through bait applications. Such tests would most closely simulate field-type control situations. Dimethoate was mixed with commercial guinea pig feed and fed to guinea pigs.

Table 13 presents the data for the feeding experiments for which the guinea pigs were prefed before the chiggers were applied. In tests number 1 and 2, guinea pigs were prefed with large amounts of dimethoate-treated feed for extended periods (517 mg/kg for 94 hours and 587.1 mg/kg for 77 hours). Upon application of the chiggers, the dimethoate-treated feed was substituted with untreated feed. In both instances the chiggers engorged to repletion: and recovery was extremely good. The fact that a build-up of dimethoate in the tissues did not occur illustrates the same type of metabolic breakdown as Kaplanis $et\ al.\ (1959)$ found occurring in cattle. The dimethoate is probably rapidly eliminated via the urine and to a lesser extent via the feces.

Similar to tests1 and 2, a large amount of pretreated food was given to guinea pig number 3 (450 mg/kg) over an extended period of time (94 hours); however, treated food was continued for an additional 29 hours with an additional 25.1 mg/kg of dimethoate being consumed. In this test none of the chiggers was recovered. All the attached chiggers were dead and desiccated in place.

Additional tests with decreasingly smaller amounts of prefed dimethoate were given to guinea pigs (numbers 4, 5, and 6), ranging from 106.3 mg/kg down to 47.2 mg/kg. In all cases chiggers did not engorge and were dead in place.

Bait tests in which the guinea pigs were prefed on dimethoater treated bait prior to application of the chiggers. Table 13.

	ć	:		•		Chiggers	gers		
Number	Dose (mg/kg)	Fed		A Attached	A Attached Engorged		B Engorged	B Attached Engorged Attached Engorged	Engorged
-	517.0	94	Treated food removed when	20	19	10	6	12	6
2	587.2	77	chiggers were applied	45	40	30	26	21	19
ю	450.0	94	Treated food	24	0	18	0	Ξ	0
	+ 25.1 475.1	29 123	continued for additional 29 hours						
4	47.2	21	Treated food	15	0	13	0	;	;
	+ 94.4	48	continued for additional 48 hours						
2	73.6	21	Treated food	33	0	42	0	;	;
	+ 63.6	<u>27</u> <u>48</u>	continued for additional 27 hours						
9	106.3	24	Treated food	28	0	35	0	;	;
	+ 147.3	24 48	continued Tor additional 24 hours						

Table 14 presents the data of chiggers killed on guinea pigs which were not given any treated feed prior to the application of the chiggers to the guinea pigs. These tests were conducted to determine what percentage of treated bait would cause complete kill of the chiggers. The treated bait ranged from 0.05 (0.5 mg dimethoate per gm of bait) to 0.2 per cent.

Guinea pigs feeding at a dose rate of 0.1 per cent or higher resulted in complete kill of the chiggers. At the 0.05 per cent level engorgement occurred with some degree of kill (15.6 per cent in one test) occurring.

Feeding Experiments of Dimethoate to Cotton Rats

Cotton rats (Sigmodon hispidus), a natural host for Eutrombicula alfreddugesi, were used for a limited number of feeding tests. Due to the difficulty in handling these rodents, a deviation in the normal recovery technique was made. The chiggers were allowed to feed a sufficient time for engorgement, 48 to 54 hours, and then the rats were sacrificed for determination of chigger mortality. A 1:1 mixture of corn and milo, treated with dimethoate, was used as the bait. In general, the bait was readily accepted by the rats. One test rat refused a 0.2 per cent preparation for the entire 48 hours, while another ate only 1.6 grams (4.8 mg dimethoate) during the same feeding period.

In systemic tests with dimethoate against the Oriental rat flea (Xenopsylla cheopis [Rothschild]), Clark (Personal communication, 1974) found, in using essentially the same formulations of corn-milo mixture

Table 14. Bait tests of dimethoate fed to guinea pigs for the control of chiggers.

Chiggers

Engorged	10	0	28
C Attached Engorged	21	17	33
Engorged	00	0	43 28
B Attached Engorged	25 19	27	31
Engorged	00	0	30 16
A Attached Engorged	22 18	24	26
Hours	48	36	41
mg/kg	253.6	110.1	47.1
Per Cent	0.2	0.1	0.02

as used in this study, that the bait was acceptable at this low concentration but not as acceptable at higher concentrations (Table 15). In his tests, the rats were offered both treated and untreated baits.

Table 16 presents the chigger mortality data for the eight rodents tested. The test dosages ranged from 0.1 to 0.4 per cent. In all the tests, in which adequate bait was consumed, virtually 100 per cent of the chiggers were killed. Only in one test at the 0.1 per cent level did any engorgement occur, with 76 per cent failing to engorge.

Carbon Dioxide Production Studies

In a normal explanation of the chigger black-plate collection techniques, invariably the first question that is asked is "Are the chiggers attracted to the plate?" In an effort to cast some light on this question, a small study on the possible response by chiggers to different wavelengths of light was initiated.

Bruce (1971) showed that the spiny rat mite (Laelaps echidnina Berlese) produced a questing movement, where the mite rests on its hind legs and moves legs I in a searching pattern, to specific wavelengths of infrared radiation (IR). Chiggers are also known to demonstrate such movement in the presence of CO₂ and when shadows are passed over them (Sasa, 1964; Sasa et al., 1957). Other physical stimulants including different intensities of light and a heated glass tube and wire, did not have any effect on the larvae of Leptotrombidium (Leptotrombidium) scutellare (Nagayo et al.) and L. (L.) akamushi (Brumpt) (Sasa et al., 1957). These tests would tend to indicate that these two species were not influenced by the presence of IR. Temperature alone is known to

Acceptability of dimethoate treated and untreated baits by cotton rats, Sigmodon hispidus. (Clark, Personal communication). Table 15.

		5	Dosage	oranis of freated and officered batt consumed Dosage (Per cent)	מובח משור מ	namen	Control
	0	0.12	0	0.24	0	0.36	An openox
Davs After		Ave	erage of 3	Average of 3 Rats Per Dosage	age		2 rats
Feeding Began	Treated	Treated Untreated	Treated	Treated Untreated	Treated	Treated Untreated	Untreated
-	2.8	8.6	2.0	7.2	2.0	9.9	9.4
2	5.5	5.7	2.6	4.5	1.3	8.3	15.8
ю	1.5	9.1	0.3	7.3	0.5	8.7	21.4
4	3.3	5.5	1.0	7.2	6.0	7.7	26.5
7	6.2	17.2	1.5	15.2	2.0	14.7	40.6
Total	18.3	47.3	7.4	41.4	6.7	46.0	113.7

Chigger morality resulting from dimethoate treated baits fed to cotton rats (Sigmodon hispidus). Table 16.

	B Mortality	Tel Cell	100	93	;	;	1	100	100	100
Results	Attached	Lagring I	39	14	1	1	1	14	10	*6
Chigger Results	A Attached Mortality Number Der Cent	100	100	57	100	100	12	100	9/	100
	Attached Aumber	15	40	21	23	34	32	18	26	24
	Insecticide Consumed Millignams	20.0	20.8	4.8	17.0	27.4	0.0	0.0	9.5	5.6
	Dose Per Cent	0.4		0.3	0.2			0.1		

*Capsule was torn loose by the rat, but 9 unengorged, dead chiggers were present.

cause increased movement of *E. splendens* (Jenkins, 1948a) and *E. alfreddugesi* (Wharton and Fuller, 1952).

Since a LIRA Model 200 Infrared Analyzer was available, these tests were set up to determine if this equipment was sensitive enough to measure CO_2 production of chiggers and to determine if CO_2 production was either increased or decreased due to changes in wavelength of light. Initial tests (not reported) indicated that although CO_2 could be detected with the equipment, sufficient levels were not produced to obtain distinct peaks during a continuous run. Thus, accumulation tests of 15 minutes were devised. This involved blocking off the chigger-holding container for 15 minutes and allowing the CO_2 to accumulate. After the 15-minute period, the CO_2 was flushed out of the container, giving a measurable quantity of CO_2 .

Different wavelengths of light were produced by using Corning glass color filters. Appendix B illustrates the transmittance of the various filters used. A 60 watt incandescent light bulb was used as the source of light, being placed approximately 7 cm from the window of the chigger-holding container. A Suprasil II filter was utilized as a window. Heat from the light was dissipated by a constant air flow in front of the window.

Two tests using approximately 300 chiggers each and one test using approximately 1,000 chiggers were conducted to compare the influence of normal light, an infrared absorbing filter (1-69) and darkness. Only in the dark tests were any differences in the ${\rm CO}_2$

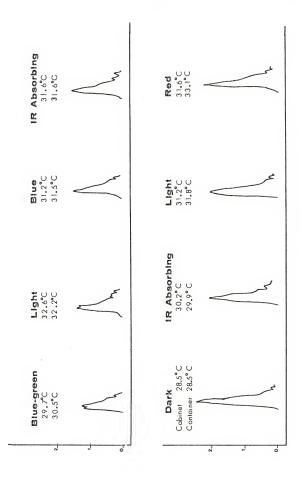
Amersil, Inc. 685 Ramsey Ave., Hillside, N. J.

peaks noted. In addition, a single series of tests using approximately 1,000 chiggers was conducted using the various filters. In addition to the IR absorbing filter, a sharp cut-off red (3-67), a blue-green (4-94) and a blue (5-58) was used. Figure 22 presents the run showing the resulting peaks of CO_2 production of the approximately 1,000 chiggers.

Two features, both agreeing with previous literature, can be noted with these sets of peaks. The first is that the peak in which the container was dark shows a slight increase in CO_2 (16 per cent). A similar increase was noted with the 300 chigger samples. This could have resulted from the questing behavior that occurs when shadows are passed over chiggers (Jones, 1950a). It should be noted that the chiggers were in a lighted situation before they were placed in darkness. The second feature of the series of peaks is that a reduction of CO_2 production occurred after the fourth test. This reduction could have been caused by deaths occurring among the chiggers, or, more probably, from fatigue. Fatigue has been previously noted to occur among chiggers (Jenkins, according to Wharton and Fuller, 1952), and as these tests had been running for over 2 hours with the chiggers active, it seems probable that the test chiggers were suffering from fatique.

The carbon dioxide production by approximately 1,000 chiggers when exposed for 15-minute intervals to light, darkness, and various color filters.

Figure 21.



CONCLUSIONS

The importance of chiggers as a pest throughout the southeastern United States is well known and can be easily demonstrated simply by walking through much of the rural countryside during late summer. The fact that chiggers transmit scrub typhus in Southeast Asia makes the control of this mite even more important. The primary purpose of this study was to determine if the use of a systemic acaricide could be effective against chiggers. Obviously, the knowledge of what chiggers are present within the general area is important in conducting any control study. This was even more so in this study as the chiggers that were to be tested were to be collected from the "wild" by the black-plate technique. In addition to reporting the results relating directly to the primary objective of the study, the chigger collection and chigger attraction (CO₂ production) studies were also recorded in this dissertation.

Regarding the chigger collections made during this study, it is apparent that little is known of the chigger distribution of the State of Florida. The six species recorded here as newly found in the State are obviously only a small fraction of the unrecorded species. An extensive chigger survey of the State should be undertaken to correct this gap in the distribution of chiggers in North America. As extensive surveys have been conducted in the western, central and to some degree in the northeastern United States, such a survey could prove important in tying together the distribution of chiggers between North and South America.

Numerous chigger species that may be found occurring here would be the same that are common to South America. The occurrence of the widespread South American chigger Eutrombicula batatas in Florida is but one example of what could exist, particularly in the subtropical habitats of south Florida. Florida with its fairly diverse habitats and with their relative ease of access would offer a unique opportunity for distribution and taxonomic studies.

Although only one insecticide, dimethoate, proved effective in the screening studies, it is obvious that it is possible that some of the tested insecticides could also be effective at increased concentrations. However, it should be pointed out that most of the insecticides used were tested at what was believed to be near the LD/50 dose to the guinea pigs. The rates used were based on previous data obtained from the United States Department of Agriculture, Insects Affecting Man Laboratory and systemic studies from other laboratories. Also numerous concentrations that proved lethal to the guinea pig or caused varying toxicity were not reported in this study. This information was used to establish a guideline for some of the insecticide concentrations utilized.

It should also be realized that even though, in the "ineffective" tests, sufficient insecticide was not obtained by the chiggers for kill, it could be possible that some of these insecticides may cause death or other effects at a later period of the chiggers' developmental cycle. These could be manifested by failure to develop, failure to produce offspring or even death. Insecticides demonstrating a variation from the normal engorgement rates may indicate that such a phenomenon could be occurring. However, as the prime objective of this study was to

determine if systemic activity of an insecticide would control the chigger, the screening tests were restricted to observations of death occurring at the time of attachment.

Although numerous systemic studies have been conducted on a large variety of insects and ticks, this study was the first attempted for the control of trombiculid larvae. The very fact that chiggers are extremely small and feed for extended periods of time, made handling and observation extremely difficult. Many of the methods developed during this study were a combination of insect and tick systemic studies. Previous techniques from feeding studies of chiggers feeding on white mice did not prove adequate for feeding chiggers on guinea pigs. The large amount of feces, urine, and hair droppings produced by the guinea pig did not allow for the relatively easy recovery of engorged larvae from pans of water placed under the guinea pigs as is used in white mice feeding. Although the initial plans were to study post-larval activity and life histories of treated and untreated chiggers, several rearing methods did not prove effective and this phase of the studies was abandoned early in the work.

The carbon dioxide studies demonstrated that the LIRA Model 200 Infrared Analyzer could be utilized in chigger studies. This study tended to support previous studies which had used visual observations and nonspecific light sources, indicating that chiggers are not affected by infrared radiation.

Although current environmental regulations would probably prevent widespread and indiscriminate usage of dimethoate or any similar acaricide that proves effective against chiggers, one could foresee the common sense usage,in restricted instances, of systemics in the control of chiggers. Bait stations accessible to chigger hosts in parks, camping areas and other recreational areas could effectively eliminate the presence of chiggers within such areas.

APPENDIX A

Chemical and toxologic information as presented by Kenaga and Allison (1969; 1970) on the insecticides utilized in screening tests against chiggers.

MANUFACTURER, MAMMALIAN TOXICITY AND

Code	Code	Names and Designations	STRUCTURAL AND EMPIRICAL FORMULAS	AVAILABILIT	Y RESIDUE TOLERANCE
C-2	1	lindane	Ç!	Cela	AO 76-200, M86, Rb60-200, D40
	2, 3	1,2,3,4,5,6-hexachlorocyclo= hexane, 99% or more gamma isomer	CITCI	Hooker Ins.	AD 500-1200, Rb300-4000 CO 50, 25(m),
	5	gamma BHC	7-1somer	1113.	D>15
	5	gamma HCH	ĊI		T EXT, 4-10
			C6H6C1E		
C-8	1	eudosulfan	ÇI	FMC Fah	AO 30-110
		6,7,8,9,10,10-hexachloro- 1,5,5a,6,9,9a-hexahydro-6,9- methano-2,4,3-benzodioxa= thiepin 3-oxide	0=50 (C12 C1	Hoech, Acar. Ins.	AD 74-130, Rb360 CO 30, D30 T EXT, 0.02-1
	5	Thiodan®	0 1		1 2311, 0.02-1
	6	Hoe 2671	01		
	6	ENT 23979	CoH6C1603S		
	6	NIA 5462	,,		
C-12	1	mirex	K-17	Allied	AO 235~702
	2	dodecachlorooctahydro- 1,3,4-metheno-1 <i>H</i> - cyclobuta[cd]pentalene	C1 ₁₂	Ins.	AD Rb800 T 0.01-0.1
	3	dodecachloropentacyclo= [5.3.0.0*.*.0*.*.0*.*]decane	C10 _{C1} 15		
	4	dodecachloropentacyclodecane			
	6	GC1283			

MAMMALIAN

INDEX CODE			Structural and Empirical Formulas	MANUFACT AVAILABIL AND US	TTY RESIDUE
M-2	2	6-methyl-2,3-quinoxaline= dithiol cyclic S,S-dithio= carbonate	N S O	Chema- gro FFB	AO 3000 AD >500 CO 50(m)
	3	6-methyl-2,3-quinoxa= linedithiol cyclic carbonate	CH3 NS	Acar.	TNR
	4	6-methyl-2-oxo-1,3- dithiolo=[4,5-b] quinoxaline	$^{\mathrm{C}_{10^{\mathrm{H}_{6}\mathrm{N}_{2}0\mathrm{S}_{2}}}$		
	5	Morestan®	10.612032		
	5	oxythioquinox			
	5	chinomethionate			
	5	quinomethionate			
	6	Bay 36205			
	6	ENT 25606			
M-7	2, 3	N'-(4-chloro-o-tolyl)- N _* N-dimethylformamidine		ÇIBA	AO 162-170, M160
	5	GALECRON®	C1 N=CH-N(CH ₃)	Acar. Ins.	AD M225 CO 250(m),
	6	8514	\ <u>_</u>		D250(m)
	6	C-8514	сн3		T. 0.5 (temp)
	6	ENT 27335			
			с ₁₀ н ₁₃ с1м ₂		
M-9		phenyl 5,6-dichloro-2- trifluoromethyl)-1- benzimidazolecarboxylate	C1 CF ₃	Fisons .1car.	AO 238-283 M ca 1600. Rb28 D>50
		5,6-dichloro-1-phenoxy= carlxonyl-2-trifluoro= methylbenzimidazole	C1 0-C-0		AD >4000 Rb >2000 AO 100 (m) T 0.75 EXT
	5	ienozaflor			
		Lovozal*			
		Tarzol®	C ₁₅ H ₇ C1 ₂ F ₃ N ₂ O ₁	?	
		Fisons NC 5016			
	6	ENT 27438			

NDEX Code	Name Code	Names and Designations	STRUCTURAL AND Empirical Formulas	Manufacturer Availability and Use	Mammalian Toxicity and Residue Tolerance
PA-20	1	dimethoate	. s o		AO 155-500,
	2	O,O-dimethyl S-(N- methylcarbamoylmethyl) phosphorodithioate	(CH30)2 - S-CH2 - NH-CH3	Cyan. Cela Fisons Monte-	M60-250, Rb300-500, Dca400 AD <150-1150
	3	O,O-dimethyl S-(N-methyl= carbamoylmethyl) phosphoro= dithioate	C5H12NO3PS2	Sumi- tomo	CO 5(m) T EXT, 0.002-2
	4	O.O-dimethyl S-a-mercapto- N-methylacetamido dithio= phosphate		Acor. Ins. Sys.	
	4	methyl dimethyl dithiophos= phoryl acetamide		Sys.	
	5	CYGON®			
	5	PERFEKTHION®			
	5	Rogor®			
	5	Roxion®			
	6	AC 12880			
	6	ENT 24650			
	6	NC-262			
	6	L 395			
PC-20	2, 3	O,O-dimethyl O-p-sulfamoyl= phenyl phosphorothicate	(0, 0) 8-0	Amer. Cyan.	AO 160, M 38-60 AD Rb >2500 CO D<15 (m)
	5	cythicate	(CH30)2P-0-((4)	00 2 (10 (111)
	5	PROBAN®	0	Ins. Sys. a.	
	6	26691	c ₈ H ₁₂ NO ₅ PS ₂	393. 4.	
PC-21	1 1	famphur	ş	Amer. Cyan.	AO 35-62, M30 AD Rb1460-509
	2, 3	O-[p-(dimethylsulfamoyl) = phenyl] O,O-dimethyl phosphorothioate	(CH ₃ 0) ₂ P-0- so ₂ N(CH ₃)	(a)	CO 1(m), D4(n T 0.1
	5	WARBEX®	CIOHIGNO _S PS ₂		
	5	Famophos	-10 16 5 2		
	6	CL 38023			
PC-38	8 2,3	phenylglyoxylonitrile oxime O,O-diethyl phosphorothioste	(C2H50)2P-0-N-C	FFB (a) Ins.	AO 8500-8800 AD >1000
	5	Valexon®	-2-5-72		
	5	Baythion®			
	5	phoxim	C ₁₂ H ₁₅ N ₂ O ₃ PS		
	6	BAY 77488	12"15"2"3"5		
		ENT 27448			

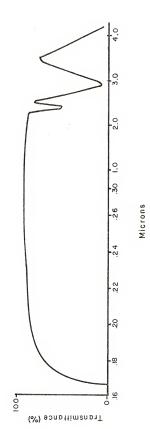
NDEX CODE			STRUCTURAL AND Empirical Formulas	Manufact Availabii and Us	ITY RESIDUE
1°H-13	2, 3	N-hydroxynaphthalimide diethyl phosphate	9 /	Chema-	AO 70-75, M50-100
	5	MARETIN®	o "	(a)	AD 140
	5	RAMETIN®	(C ₂ H ₅ O) ₂ P-O-N	FFB (a)	T NR
	6	BAY S-940	2 3 12 1	Sys. a.	
	6	BAY 9002	<u>l</u> ('>	
	6	BAY 25820	° \/	/	
	6	ENT 25567	C ₁₆ H ₁₆ NO ₆ P		
PH-16	2, 3	O,O-diethyl O-(3,5,6- trichloro-2-pyridyl) phosphorothioate	s CI	Dow Acar.	AO 97-276, R51000-2000 AD R52000
	5	Dursban®	(C ₂ H ₅ O) ₂ P-O-	Ins.	T NR
	6	Dowco® 179	(C2150)21-0-0 N / CI		
	6	ENT 27311			
			C9H11C13NO3PS		
H-18	1	diasinon			
		O,O-diethyl O-(2-isopropyl- 4-methyl-6-pyrimidyl) phosphorothioate	(C2H2O)2P-O 1 N= CH(CH3)2	Geigy Acar. Ins.	AO 66-600, M80-135, Rb130-143
		O,O-diethyl O-(2-isopropyl- 6-methyl-4-pyrimidinyl) phosphorothioate	CH ₃	Ins.	AD 379-1200, Rb4000 CO 1, D 0.75 T 0-60
		O.O-diethyl O-(2-isopropyl- 6-methyl-4-pyrimidyl) thiophosphate	C12H21N2O3PS		
		Diazinon®			
	5	Basudin			
	6 (G-24480			
		•			
5	2,3	benzyl benzoate	C-OCH ₃ -C	Mon- santo	AO 1700, M1400, Rb1800 T NR
				RP	
			C14H12O2		

INDEX	NAME Code	Names and Designations	STRUCTURAL AND EMPIRICAL FORMULAS	Manufactu Availabili and Use	
X-9	2, 3	4-(methylthio)3,5-xylyl methylcarbamate	C11H12N052	Chema- AD >200	AO 130-135 AD >200
	5	MESUROL®			CO 10(m)
	5	mercaptodimethur			
	5	methiocarb			
	6	BAY 9026			
	6	BAY 37344			
	6	ENT-25726			
X-15	1	carbaryl	0	Union	AO 307-986.
	2	1-naphthyl methylcarbamate	o-c-nh-ch,	Carb.	D>759, Rb710
	3	1-naphthyl N-methylcarbamate	~ J		AD >500->4000, Rb>2000
	4	a-naphthyl N-methylcarbamate		Ins.	CO 200, D200-400
	5	SEVIN®			T EXT, 0-100
	6	7744	C 12H I NO		
			12 11 2		

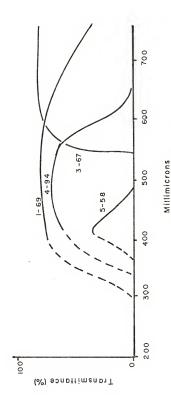
APPENDIX B

Per cent transmission of filters utilized in ${\rm CO_2}$ studies.

Per Cent Transmission of Suprasil Filter^l



'Amersil, Inc.; 685 Ramsey Avenue; Hillside, N. J.



Per cent transmission of four corning color filters used in carbon dioxide studies.

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BIOGRAPHICAL SKETCH

Alexander Louis Dohany was born in Lakeland, Florida, on 9 December 1941 to Andrew and Daisy Dohany. He graduated from Lakeland Senior High School in 1959. He attended Newberry College, Newberry, South Carolina, for his freshman year, and then transferred to the University of Florida, Gainesville, Florida, from which he received his Bachelor of Science in Agriculture, majoring in Entomology, in 1963. In 1965, he completed a Master of Science in Forestry degree from the University of Washington, Seattle, Washington, and entered the Medical Service Corps of the United States Army as a Second Lieutenant.

After completing the basic M.S.C. course, he was assigned to the Fourth U. S. Army Medical Laboratory, Fort Sam Houston, Texas, as a Medical Entomologist. In 1966 he became Officer-in-Charge of a Preventive Medicine Control team in northcentral Thailand and was transferred to the 712th Preventative Medicine Unit when it arrived in the country.

From 1967 through 1970, Captain Dohany was assigned to the U. S. Army Medical Research Unit at the Institute for Medical Research, Kuala Lampur, Malaysia, and conducted medical research on the vectors of scrub typhus and malaria. After completion of the M.S.C. Advanced Officer Course in 1971, he returned to the University of Florida to begin work on his Doctor of Philosophy degree.

Captain Dohany is married to the former Jean Louis Kaiser and has one son, Timothy Alan Dohany. He is a certified Medical Entomologist with the Entomological Society of America and is a member of the Malaysian Society of Parasitology and Tropical Medicine.

I certify that I have read this study and that in my opinion it conforms to acceptable standards of scholarly presentation and is fully adequate, in scope and quality, as a dissertation for the degree of Doctor of Philosophy

H. L. Cromroy, Chairman

Professor of Entomology and Nematology

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Assistant Professor of Entomology and Nematology

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This dissertation was submitted to the Graduate Faculty of the College of Agriculture and to the Graduate Council, and was accepted as partial fulfillment of the requirements for the degree of Doctor of Philosophy.

December, 1974

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